

## **REPORT**

### **Study Title:**

Determination of *n*-Octanol/Water Partition Coefficient of K32

**Ricerca Study Number: 035238**

**Ricerca Document Number: 035238-1**

### **Data Requirement:**

US EPA OPPTS Test Guidelines 830.7550 Partition Coefficient  
(*n*-octanol/water), Shake Flask Method (August 1996)

### **Author:**

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### **Study Completion Date:**

14-Jun-2017

#### **Testing Facility:**

AgChem Product Development  
Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord OH 44077

#### **Study Sponsor:**

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Report/K32 *n*-Octanol/Water Partition Coefficient  
Document Number: 035238-1

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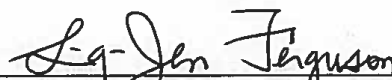
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Report/K32 *n*-Octanol/Water Partition Coefficient  
Document Number: 035238-1

## COMPLIANCE STATEMENT

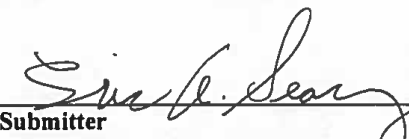
The study reported herein, "Determination of *n*-Octanol/Water Partition Coefficient of K32," Ricerca Document Number 035238-1, was conducted and reported in compliance with the Good Laboratory Practice Regulations set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.

  
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June 14, 2017  
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Date

  
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Eric Searcy, Sponsor Representative  
Koch Agronomic Services, LLC

6/14/2017  
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## QUALITY ASSURANCE STATEMENT

The Ricerca Quality Assurance Unit has performed inspections on the study, "Determination of *n*-Octanol/Water Partition Coefficient of K32," Ricerca Study Number 035238. The results of these inspections, including any findings or observations, were reported to the Study Director and Management for appropriate corrective actions on the dates listed below:

Phase of Study Inspected	Dates Inspected	Dates Reported to Study Director	Dates Reported to Management
Protocol	Jan 23, 2017	Jan 23, 2017	Jan 23, 2017
In-Study	Mar 21, 2017	Mar 21, 2017	Mar 21, 2017
Data and Report	Apr 18, 2017	Apr 19, 2017	Apr 19, 2017

  
Ann O'Leary, Ph.D.  
Ricerca Biosciences Quality Assurance

  
Date



Report/K32 *n*-Octanol/Water Partition Coefficient  
Document Number: 035238-1

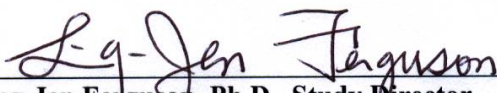
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## APPROVALS


Study Title: Determination of *n*-Octanol/Water Partition Coefficient of K32

Document Number: 035238-1

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June 14, 2017  
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## ABSTRACT

The shake flask method was employed to determine the *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32. The phosphorus (P) content in the two phases (*n*-octanol and water) was quantitated by ICP-AES. The *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32 was calculated from the measured P concentration in the *n*-octanol phase divided by the measured P concentration in the aqueous phase.

In the preliminary study, samples with the *n*-octanol/water ratio at 1:1 in duplicates (total 2 samples) were prepared and analyzed. The  $P_{ow}$  of K32 was calculated to be 4.0. The log  $P_{ow}$  is 0.6 and the recoveries of phosphorus in both samples are 103%.

In the definitive study, samples with *n*-octanol/water ratios at 1:1, 2:1, and 1:2 in duplicates (total 6 samples) were prepared and analyzed. The  $P_{ow}$  of K32 was calculated to be 4.3 (*n*-octanol/water ratio 1:1,  $n = 2$ ), 3.1 (*n*-octanol/water ratio 2:1,  $n = 2$ ), and 5.0 (*n*-octanol/water ratio 1:2,  $n = 2$ ).

- The overall  $P_{ow}$  (average  $\pm$  standard deviation) is  $4.1 \pm 0.9$  ( $n = 6$ ).
- The overall log  $P_{ow}$  is  $0.6 \pm 0.1$ , which is within the guideline requirement of  $\pm 0.3$  log units.

The recoveries of phosphorus in all samples from the definitive studies ranged from 97.3-105%.



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## **OBJECTIVE/PURPOSE**

The purpose of this study was to determine the *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32. K32 is a new product candidate developed by Koch Agronomic Services, LLC. K32 is a mixture of reaction products that incorporate *N*-(*n*-butyl) thiophosphoric triamide (NBPT), the active ingredient in AGROTAIN®, a nitrogen stabilizer product currently marketed by Koch Agronomic Services, LLC. The study was conducted to meet the registration requirement for US EPA, US EPA Guideline OPPTS 830.7550 Partition Coefficient (*n*-Octanol/Water), Shake Flask Method, (Product Properties Test Guidelines).

## **STUDY INFORMATION**

### ***STUDY TITLE***

Determination of *n*-Octanol/Water Partition Coefficient of K32

### ***STUDY NUMBER***

035238

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### ***SCHEDULE OF EVENTS***

Study Initiation Date:	February 1, 2017
Experimental Start Date:	February 8, 2017
Experimental Termination Date:	March 23, 2017
Study Completion Date:	June 14, 2017

### ***RETENTION OF DATA***

Upon completion of the study, the complete study file, including all original raw data, protocol and amendment one, and final report, will be submitted to the Ricerca Biosciences, LLC archives. All non-study-specific raw data (e.g., instrument logs) are archived at Ricerca Biosciences, LLC.

### ***CONDUCT OF THE STUDY***

The study was conducted at the Ricerca Biosciences, LLC AgChem Product Development Department Laboratories according to the Ricerca Biosciences, LLC protocol “Determination of *n*-Octanol/Water Partition Coefficient of K32” (Ricerca Document Number 035238-0), and amendment one (Ricerca Document Number 035238-0-1) ([Appendix A](#)).

Personnel involved with the study were:

Ling-Jen Ferguson	Scientist
Richard L. Shea	Associate Scientist I
Kenneth Furlong	Associate Scientist III

## MATERIALS AND METHODS

### *TEST SUBSTANCE*

- **K32**

Composition:	Reaction products of NBPT with urea and formaldehyde
Batch/Lot Number:	55700-30-13
Analyzed Concentration:	NBPT 20.04 wt%; Reaction product mixtures ~80 wt%
% Phosphorus Content	12.4%
Manufactured by:	Ricerca Biosciences
Date of Manufacture:	July 20, 2016
Appearance:	Off-white to pale yellow gel
Storage:	Refrigerated

### *STORAGE*

K32 (Lot No. 55700-30-13) was synthesized by Ricerca (Study No. 034689) and received for studies on 8/19/2016. All preparations of the test substance were uniquely identified and stored at ambient temperature.

Upon completion of the study any portion of the test substance not utilized in the study will remain in storage at Ricerca Biosciences unless otherwise directed by the Sponsor.

### *CHARACTERIZATION OF TEST SUBSTANCE*

GLP characterization of K32 was performed under Ricerca Study No. 035469. A certificate of analysis (CoA) of K32 from Ricerca Study No. 035469 is included in [Appendix B](#). The Sponsor assumed responsibility of retention of a sample of test material, as specified in 40 CFR 160.195.

### *SHAKE FLASK METHOD*

The determination of  $P_{ow}$  described here follows the shake flask method detailed in OPPTS Guideline 830.7550. To determine a partition coefficient, equilibrium between all interacting components of the system must be achieved, and the concentrations of the substances dissolved in the two phases must be determined. This is accomplished by the thorough mixing of the two phases followed by their separation in order to determine the equilibrium concentration for the substance being examined.

The Nernst partition law applies only at constant temperature, pressure, and pH for dilute solutions. It strictly applies to a pure substance dispersed between two pure solvents. If several different solutes occur in one or both phases at the same time, this may affect the results. Dissociation or association of the dissolved molecules results in deviations from the Nernst partition law. Such deviations are indicated by the fact that the partition coefficient becomes dependent upon the concentration of the solution. Because of the

multiple equilibria involved, this test method should not be applied to ionizable compounds without corrections being made. The use of buffer solutions in place of water should be considered for such compounds.

The sensitivity of the method is determined by the sensitivity of the analytical procedure, which should be sufficient to permit the assessment of values of  $P_{ow}$  up to  $10^5$  when the concentration of the solute in either phase is not more than 0.01 mol/L. The substance being tested must not be water insoluble (mass concentration  $p > 10^{-6}$  g/L).

#### ***REAGENTS***

- Water: RICCA Chemical (Arlington, TX) ASTM Type I, ASTM Type II, Double Distilled, ACS Reagent Grade, Cat. No.9150-1 (referred as “water” in this report)
- *n*-Octanol: Fisher Certified Grade
- Water: House Deionized Water (referred as “deionized water or DIH<sub>2</sub>O” in this report)
- Acetonitrile: Fisher Optima Grade
- 1000 µg/mL NIST-traceable P std reference solution (CPI 4400-1000391)
- 1000 µg/mL NIST-traceable Y std reference solution (CPI 4400-1000671)
- Sulfuric Acid, Fisher Trace Metal Grade A510-P212
- Nitric acid, Fisher Trace Metal Grade A509-P212

#### ***EQUIPMENT***

- Incubator/shaker maintained at 25 °C (Stuart Orbital Shaker LLS1: speed set at 130 rpm max time 90 min; the solvents were mixed by hand-shaking each time before re-starting the shaker.)
- Analytical Balance
- Centrifuge: Sorvall Legend X1R (the temperature set at 25 °C)
- Perkin Elmer Optima 3000 Dual-View Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES)
- Standard glass lab ware (flask, graduated cylinders, etc.)

#### ***PRE-SATURATION OF THE SOLVENTS***

A 1-L glass bottle (Bottle A) was filled with 900 mL of *n*-octanol and 100 mL of water. Another 1-L glass bottle (Bottle B) was filled with 900 mL of water and 100 mL of *n*-octanol. Both bottles were placed on a shaker in an incubator ( $25 \pm 1$  °C) for 24 hours.

The *n*-octanol saturated with water was poured from “Bottle A” into a glass bottle. Using a 1-L separatory funnel, the water saturated with *n*-octanol was collected from “Bottle B” into another glass bottle. The prepared solvents were stored at ambient temperature in the lab for use in this study.

#### ***PRELIMINARY AND DEFINITIVE STUDY OF THE PARTITION COEFFICIENT***

A preliminary partition experiment was conducted to determine the partition equilibration of K32 using the shake flask method. Samples were prepared in duplicate at a solvent

ratio of 1:1 (*n*-octanol saturated with water: water saturated with *n*-octanol). The phosphorus content in both phases (*n*-octanol and water) were analyzed by ICP-AES.

A definitive partition experiment was conducted to determine the partition equilibration of K32 using the shake flask method. Samples were prepared in duplicate at a solvent ratio of 1:1, 2:1, and 1:2 (*n*-octanol saturated with water: water saturated with *n*-octanol). The phosphorus content in both phases (*n*-octanol and water) were analyzed by ICP-AES. The pH of the aqueous phase was measured using a pH meter.

### Preparation of K32 Stock Solutions in *n*-Octanol Saturated with Water

#### Preliminary Study

- **K32 Stock Solution** (250 µg/mL K32 in *n*-octanol saturated with water, not purity corrected). K32 (28.0 mg) was weighed into a 250-mL glass bottle with a Teflon-lined cap and 112 mL of *n*-octanol saturated with water was added. The mixture was sonicated to give a clear solution (final concentration: 250 µg/mL).

#### Definitive Study

- **K32 Stock Solution** (250 µg/mL K32 in *n*-octanol saturated with water, not purity corrected). K32 (53.6 mg) was weighed into a 250-mL glass bottle with a Teflon-lined cap and 214.4 mL of *n*-octanol saturated with water was added. The mixture was sonicated to give a clear solution (final concentration: 250 µg/mL).

### Procedure

- Using glass pipets the following were set up in 50-mL Nalgene PPCO (Polypropylene Copolymer) Centrifuge Tubes (can only hold up to 42 mL), as detailed in Tables 1 and 2:

**Table 1: Sample Set-up for K32 Partition Coefficient Preliminary Study**

Sample	Water saturated with <i>n</i> -Octanol (mL)	250 µg/mL K32 in <i>n</i> -octanol saturated with water* (mL)	<i>n</i> -Octanol saturated with water (mL)	Ratio of <i>n</i> -Octanol vs. Water
#1	21	14	7	1:1
#2	21	14	7	1:1

\*not corrected for purity

**Table 2: Sample Set-up for K32 Partition Coefficient Definitive Study**

Sample	Water saturated with <i>n</i> -Octanol (mL)	250 µg/mL K32 in <i>n</i> -octanol saturated with water* (mL)	<i>n</i> -Octanol saturated with water (mL)	Ratio of <i>n</i> -Octanol vs. Water
#1	21	14	7	1:1
#2	21	14	7	1:1
#3	14	14	14	2:1
#4	14	14	14	2:1
#5	28	14	0	1:2
#6	28	14	0	1:2

\*not corrected for purity

- The centrifuge tubes were shaken by hand. The centrifuge tubes were quickly rotated (100 rotations in 5 min) through 180° about the transverse axis so that any trapped air rises through the two phases.
- The centrifuge tubes were centrifuged at 25 °C (Sorvall Legend X1R Centrifuge S/N 41371804, 2000 rpm, 20 min)
- An aliquot of each of the two phases were removed as much as possible from each tube for ICP-AES measurement. The *n*-octanol phase at the top was removed using a glass pipet to a glass vial. The aqueous phase in the bottom was sampled by a glass syringe with a removable needle. The syringe was initially partially filled with air. Air was gently expelled while inserting the needle through the *n*-octanol layer. An adequate volume of aqueous phase was withdrawn into the syringe. The syringe was detached and attached from the needle (the top of the needle above the solvent level) a few times to remove the aqueous phase to a glass vial.

**An identical set-up as in Table 2 was repeated in the definitive study and the aqueous phase was separated for pH measurement.**

- The pH of the water used and the aqueous phase for each of the samples in the definitive study was measured.

#### **ICP-AES ANALYSIS**

ICP-AES using an internal standard methodology was employed to evaluate the phosphorus (P) contents in the test solutions.

#### **Sample Treatment prior to ICP-AES Analysis**

Test and QC samples were treated as follows prior to ICP-AES Analysis. An aliquot (8 mL) was placed in a 200-mL tall-form beaker. The beaker was placed in a warm hotplate in a fume hood to evaporate to near dryness. Sulfuric Acid (3 mL) was added to the beaker, which was then covered with a watch glass and placed on a hotplate. The temperature of the hotplate was increased slowly until the sample began to fume, and then small aliquots of

nitric acid was added to ensure complete sample digestion. A total of 0.75 mL of nitric acid was added. The temperature of the hotplate was eventually raised to “high”. After digestion was completed and all nitric acid fumes were evolved, the beaker was removed from the hotplate and allowed to cool. The residue was dissolved in house deionized water and transferred to a 50-mL polypropylene tube. The sample was diluted to 30 mL with deionized water before ICP-AES analysis.

### **Preparation of QC Samples**

Preparation of K32 fortification solution by weighing 0.0550 g of K32 into a 50-mL volumetric flask and diluted to volume with DIH<sub>2</sub>O-ACN (1:1, v:v). DIH<sub>2</sub>O-ACN (1:1, v:v) was prepared by mixing 50 mL of deionized water and 50 mL of ACN.

The two QC samples in water saturated with *n*-octanol were prepared by spiking 1 mL each of the K32 fortification solution to 8 mL each of the two aqueous test samples from the preliminary study. The samples were then treated following the procedure described in **Sample Treatment prior ICP-AES Analysis**. The P content present in the non-fortified test samples were subtracted from the P content present in the fortified samples and the recoveries were calculated.

The QC sample in *n*-octanol saturated with water was prepared by spiking 1 mL of the K32 fortification solution to 8 mL of *n*-octanol saturated with water. The sample was then treated following the procedure described in **Sample Treatment prior ICP-AES Analysis** to determine P content and calculate recovery.

### **Preparation of Calibration Standards**

100 µg/mL P Stock Solution – 10 mL of a 1000 µg/mL NIST-traceable P std reference solution was pipetted into a 100-mL volumetric flask, and diluted with deionized water to volume.

Calibration standards containing 0, 4, 8, and 12 µg/mL of P in deionized water were prepared by pipetting 0, 4.00, 8.00, and 12.00 mL of the 100 µg/mL P stock solution to four 100-mL volumetric flasks (contained 50 mL deionized water each and placed in an ice bath), and deionized water was added to volume.

### **Preparation of Internal Standard**

10 µg/mL Yttrium Internal Standard Reference Solution - 1 mL of a 1000 µg/mL NIST-traceable Y std reference solution was pipetted into a plastic bottle, and diluted with deionized water to 100 mL.

### **Sample Analysis**

Samples were analyzed against the calibration curves on the Perkin Elmer Optima 8300 Dual-View Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). The sample solution (or the calibration standard solution) and the internal standard solution were pumped into the mixing block simultaneously through tubing of different diameters. The mixed sample/internal standard solution is then pumped through a single tube into the instrument sample introduction system. The mixing block will automatically dilute the 10 µg/mL Y to approximately 1 µg/mL in each standard and sample solution as it is aspirated into the torch. The samples and calibration standards were analyzed in “axial” mode, three replicates, high purge gas flow. Three independent wavelengths (213.617 nm, 214.914 nm, and 178.221 nm) were evaluated. The ICP-AES automatically corrected the P concentration against the Y concentration. The LOQ (in µg/mL) was determined by injection of the calibration blank, 12 replicates (standard deviation x 10 x 3.75 [~Dilution Factor]).

### ***PH MEASUREMENT***

The pH of the water used and the aqueous phase for each of the samples in the definitive study was measured by Accumet AB 150 pH/mV meter.

### ***STABILITY AT TEST CONDITIONS***

The *n*-octanol/water partition coefficients determined in this study are solely based on the phosphorus content measured by ICP-AES regardless of K32 components present and therefore, the stability study of K32 at test conditions was not performed.



## RESULTS AND DISCUSSION

### PRELIMINARY STUDY

The ICP-AES was calibrated with P std reference solutions at three wavelengths (213.617 nm, 214.914 nm, and 178.221 nm) and the result is shown in Table 3. The detector calibration was found to be linear over the range 4 to 12 µg/mL of P in deionized water.

**Table 3: ICP-AES Calibration Results (Preliminary Study)**

Wavelength (nm)	Correlation coefficient	LOQ (µg/mL P)	8 µg/mL P std
Preliminary Study Water Phase Analysis			
213.617	0.999867	0.4	7.893
214.914	0.999895	0.5	7.969
178.221	0.999996	2.8	7.912
Preliminary Study Octanol Phase Analysis			
213.617	0.999974	0.3	7.906
214.914	0.999988	0.4	7.938

Based on the evaluation, all three wavelengths were appropriate for analysis except that 178 nm was less sensitive. The reported test sample analysis results in Table 4 are the average values from all three wavelengths for water phase analysis and the average values from 213 nm and 214 nm for octanol phase analysis. The QC sample recoveries are based on the average values from 213 nm and 214 nm. The wavelength 178 nm was not used in all analyses due to lower sensitivity.

The phosphorus (P) content in the two phases (*n*-octanol and water) of the two samples in the preliminary study was quantitated by ICP-AES. Table 4 shows the measured P content in the two phases and the calculation of  $P_{ow}$  and  $\log P_{ow}$ . The *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32 was calculated from measured P concentration in the *n*-octanol phase divided by measured P concentration in the aqueous phase and was determined to be 4.0 (*n*-octanol/water ratio 1:1, *n* = 2). The  $\log P_{ow}$  is 0.6.

**Table 4: Phosphorus Content in *n*-Octanol and Aqueous Phases and Calculation of  $P_{ow}$  (Preliminary Study)**

Sample	Ratio of <i>n</i> -Octanol vs. Water	ICP-AES Measured Phosphorus Content (µg/mL P) in		$P_{ow}$	Mean $P_{ow}$	Log Mean $P_{ow}$
		<i>n</i> -Octanol Phase	Aqueous Phase			
#1	1:1	17	4.3	4.0	4.0	0.6
#2	1:1	17	4.3	4.0		

The recoveries of phosphorus in both samples from the preliminary study were 103%. Table 5 shows the calculation of the recoveries. The recoveries of the QC samples prepared from spiking known amounts of K32 in *n*-octanol saturated with water or water saturated with *n*-octanol range 87-111%.

**Table 5: Recoveries (Preliminary Study)**

Sample	Total K32 Introduced <sup>a</sup> (µg)	Total P Introduced <sup>b</sup> (µg)	Total P in <i>n</i> -Octanol Phase <sup>c</sup> (µg)	Total P in Aqueous Phase <sup>c</sup> (µg)	Total P in <i>n</i> -Octanol + Aqueous Phase (µg)	Recovery <sup>d</sup>
#1	3500	434	357	90.3	447.3	103%
#2	3500	434	357	90.3	447.3	103%
QC in <i>n</i> -octanol saturated with water <sup>e</sup>						109%
QC in water saturated with <i>n</i> -octanol <sup>f</sup>						87%, 111%

<sup>a</sup>Total K32 introduced: 250 µg/mL x 14 mL = 3500 µg

<sup>b</sup>Total P introduced: 3500 µg x 12.4% = 434 µg (P content of K32 = 12.4%)

<sup>c</sup>Total P in *n*-octanol or aqueous phase: P concentration (µg/mL measured by ICP-AES, Table 4) x Volume (mL, Table 1)

<sup>d</sup>Recovery = (Total P in *n*-Octanol + Aqueous phase)/(Total P Introduced) x 100%

<sup>e</sup>The QC sample in *n*-octanol saturated with water was prepared by spiking 1 mL of a K32 fortification solution (0.0550 g of K32 in 50 mL of DIH<sub>2</sub>O-ACN (1:1, v:v)) to 8 mL of *n*-octanol saturated with water.

<sup>f</sup>The two QC samples in water saturated with *n*-octanol were prepared by spiking 1 mL each of a K32 fortification solution (0.0550 g of K32 in 50 mL of DIH<sub>2</sub>O-ACN (1:1, v:v)) to 8 mL each of the two aqueous test samples from the preliminary study.

## DEFINITIVE STUDY

The ICP-AES was calibrated with P std reference solutions at two wavelengths (213.617 nm and 214.914 nm) and the result is shown in Table 6. The detector calibration was found to be linear over the range 4 to 12 µg/mL of P in deionized water.

**Table 6: ICP-AES Calibration Results (Definitive Study)**

Wavelength (nm)	Correlation coefficient	LOQ (µg/mL P)	8 µg/mL P std
213.617	0.999833	0.4	8.237
214.914	0.999890	0.7	8.199

There were spectral background interferences with 214 nm and the reported test sample analysis results in Table 7 (below) are the values from 213 nm.

The phosphorus (P) content in the two phases (*n*-octanol and water) of the six samples in the definitive study was quantitated by ICP-AES. Table 7 shows the measured P content in the two phases and the calculation of P<sub>ow</sub> and log P<sub>ow</sub>. The *n*-octanol/water partition coefficient (P<sub>ow</sub>) of K32 was calculated from measured P concentration in the *n*-octanol phase divided by measured P concentration in the aqueous phase to be 4.3 (*n*-octanol/water ratio 1:1, n = 2), 3.1 (*n*-octanol/water ratio 2:1, n = 2), and 5.0

(*n*-octanol/water ratio 1:2, *n* = 2). The overall  $P_{ow}$  (average  $\pm$  standard deviation) is  $4.1 \pm 0.9$  (*n* = 6). The overall log  $P_{ow}$  is  $0.6 \pm 0.1$ .

**Table 7: Phosphorus Content in *n*-Octanol and Aqueous Phases and Calculation of  $P_{ow}$  (Definitive Study)**

Sample	Ratio of <i>n</i> -Octanol vs. Water	ICP-AES Measured Phosphorus Content ( $\mu\text{g/mL P}$ ) in		$P_{ow}$	Mean $P_{ow}$	Log Mean $P_{ow}$
		<i>n</i> -Octanol Phase	Aqueous Phase			
#1	1:1	16	4.1	3.9	4.3	0.6
#2	1:1	18	3.8	4.7		
#3	2:1	14	4.5	3.1	3.1	0.5
#4	2:1	14	4.5	3.1		
#5	1:2	22	4.7	4.7	5.0	0.7
#6	1:2	22	4.2	5.2		
Average				4.1	4.1	0.6
Standard Deviation				0.9	0.9	0.1

The recoveries of phosphorus in all 6 samples from the definitive study range 97.3-105%. Table 8 shows the calculation of the recoveries.

**Table 8: Recoveries (Definitive Study)**

Sample	Total K32 Introduced <sup>a</sup> ( $\mu\text{g}$ )	Total P Introduced <sup>b</sup> ( $\mu\text{g}$ )	Total P in <i>n</i> -Octanol Phase <sup>c</sup> ( $\mu\text{g}$ )	Total P in Aqueous Phase <sup>c</sup> ( $\mu\text{g}$ )	Total P in <i>n</i> -Octanol + Aqueous Phase ( $\mu\text{g}$ )	Recovery <sup>d</sup>
#1	3500	434	336	86.1	422	97.3%
#2	3500	434	378	79.8	458	105%
#3	3500	434	392	63.0	455	105%
#4	3500	434	392	63.0	455	105%
#5	3500	434	308	132	440	101%
#6	3500	434	308	118	426	98.1%

<sup>a</sup>Total K32 introduced:  $250 \mu\text{g/mL} \times 14 \text{ mL} = 3500 \mu\text{g}$

<sup>b</sup>Total P introduced:  $3500 \mu\text{g} \times 12.4\% = 434 \mu\text{g}$  (P content of K32 = 12.4%)

<sup>c</sup>Total P in *n*-octanol or aqueous phase: P concentration ( $\mu\text{g/mL}$  measured by ICP-AES, Table 6)  $\times$  Volume (mL, Table 2)

<sup>d</sup>Recovery = (Total P in *n*-Octanol + Aqueous phase)/(Total P Introduced)  $\times$  100%

#### ***pH MEASURE OF THE AQUEOUS PHASE***

The pH of the water used and the aqueous phase for each of the samples in the definitive study was measured by Accumet AB 150 pH/mV meter. The pH meter was calibrated

with a pH 4 buffer, a pH 7 buffer, and a pH 10 buffer (slope = 92.0%). Table 9 summarizes the result.

**Table 9: pH of Aqueous Phase**

Sample	pH
Water used	8.53
Sample #1 Aqueous Phase	8.34
Sample #2 Aqueous Phase	8.35
Sample #3 Aqueous Phase	8.35
Sample #4 Aqueous Phase	8.34
Sample #5 Aqueous Phase	8.46
Sample #6 Aqueous Phase	8.45

## CONCLUSION

The shake flask method was employed to determine the *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32. Samples with the *n*-octanol/water ratio at 1:1 in duplicates (total 2 samples) were prepared and analyzed in the preliminary study. The phosphorus (P) content in the two phases (*n*-octanol and water) was quantitated by ICP-AES. The *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32, calculated from the measured P concentration in the *n*-octanol phase divided by the measured P concentration in the aqueous phase, was 4.0 (*n*-octanol/water ratio 1:1, *n* = 2). The log  $P_{ow}$  is 0.6 and the recoveries of phosphorus in both samples are 103%.

In the definitive study, samples with *n*-octanol/water ratios at 1:1, 2:1, and 1:2 in duplicates (total 6 samples) were prepared and analyzed. The phosphorus (P) content in the two phases (*n*-octanol and water) was quantitated by ICP-AES. The *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32 was calculated from the measured P concentration in the *n*-octanol phase divided by the measured P concentration in the aqueous phase to be 4.3 (*n*-octanol/water ratio 1:1, *n* = 2), 3.1 (*n*-octanol/water ratio 2:1, *n* = 2), and 5.0 (*n*-octanol/water ratio 1:2, *n* = 2).

- The overall  $P_{ow}$  (average  $\pm$  standard deviation) is  $4.1 \pm 0.9$  (*n* = 6).
- The overall log  $P_{ow}$  is  $0.6 \pm 0.1$ , which is within the guideline requirement of  $\pm 0.3$  log units.

The recoveries of phosphorus in all samples from the definitive studies ranged from 97.3-105%.

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## **APPENDIX A: PROTOCOL AND AMENDMENT ONE**

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## **PROTOCOL**

### **Study Title:**

Determination of *n*-Octanol/Water Partition Coefficient of K32

**Ricerca Document Number:** 035238-0

**Ricerca Study Number:** 035238

### **Data Requirements:**

US EPA OPPTS Test Guidelines 830.7550 Partition Coefficient (n-octanol/water),  
Shake Flask Method (August 1996)

### **Testing Facility:**

AgChem Product Development  
Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord OH 44077

### **Study Sponsor:**

Koch Agronomic Services, LLC  
2883 Miller Road  
Decatur GA 30035



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## INTRODUCTION

K32 is a new product candidate developed by Koch Agronomic Services, LLC. K32 is a mixture of reaction products that incorporate N-(n-butyl) thiophosphoric triamide (NBPT), the active ingredient in AGROTAIN®, a nitrogen stabilizer product currently marketed by Koch Agronomic Services, LLC.

This study will develop data to help predict the potential for bioaccumulation of the test substance in living tissue or other organic matter by measurement of its *n*-octanol/water partition coefficient ( $P_{ow}$ ).

### **PURPOSE**

The purpose of this study is to determine the *n*-octanol/water partition coefficient ( $P_{ow}$ ) of K32. The study will be conducted to meet the registration requirement for US EPA, US EPA Guideline OPPTS 830.7550 Partition Coefficient (*n*-Octanol/Water), Shake Flask Method (Product Properties Test Guidelines).

## STUDY INFORMATION

### **STUDY TITLE**

Determination of n-Octanol/Water Partition Coefficient of K32

### **RICERCA STUDY NUMBER**

035238

### **SPONSOR**

Koch Agronomic Services, LLC  
2883 Miller Road  
Decatur GA 30035

### **SPONSOR REPRESENTATIVE**

Eric Searcy  
Product Regulatory Manager  
Koch Agronomic Services, LLC  
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Decatur, GA 30035  
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### **TESTING FACILITY**

Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord, OH 44077-1000



#### ***STUDY DIRECTOR***

Ling-Jen Ferguson, Ph.D.  
Ricerca Biosciences, LLC  
AgChem Product Development  
7528 Auburn Road  
Concord, OH 44077  
Phone: 440-357-3672  
Email: ling-jen.ferguson@ricerca.com

#### ***REGULATORY COMPLIANCE***

This study will be conducted in accordance with the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR 160.

#### ***SCHEDULE OF EVENTS***

Proposed Experimental Starting Date: January 2017  
Proposed Experimental Completion Date: February 2017

The actual starting and completion dates will be documented in the final report.

### **MATERIALS AND METHODS**

#### ***STORAGE, DISTRIBUTION, CHARACTERIZATION, AND RETENTION***

The Sponsor has supplied the test substance. The test substance will be handled throughout the testing to preclude the possibility of contamination, deterioration, or damage. The Sponsor will assume the responsibility of retention of a sample of the test substance, as specified in 40 CFR 160.195.

Upon completion of the study any portion of the test substance not utilized in the study will remain in storage at Ricerca Biosciences unless otherwise directed by the Sponsor.

#### ***TEST SUBSTANCE***

##### **• K32**

Composition:	Reaction products of NBPT with urea and formaldehyde
Batch/Lot Number:	55700-30-13
Analyzed Concentration:	NBPT 20.04 wt%; Reaction product mixtures ~80 wt%
% Phosphorus Content	12.4%
Manufactured by:	Ricerca Biosciences
Date of manufacture:	July 20, 2016
Appearance:	Off-white to pale yellow gel
Storage:	Refrigerated



K32 (Lot No. 55700-30-13) was synthesized by Ricerca (Study No. 034689). GLP characterization of K32 was performed under Ricerca Study No. 035469.

#### ***STABILITY OF THE TEST SUBSTANCE AT TEST CONDITIONS***

An Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) will be the analytical methodology for this study, to determine the phosphorus content in the aqueous phase of the K32 octanol-water test samples. The *n*-octanol/water partition coefficients determined in this study will be solely based on the phosphorus content measured by ICP-AES regardless of K32 components present and therefore, the stability study of K32 at test conditions will not be performed.

### **JUSTIFICATION FOR SELECTION OF TEST SYSTEM**

The test procedures have been selected to comply with US EPA registration requirement: United States Environmental Protection Agency Product Properties Test Guidelines, 1996.

### **EXPERIMENTAL DESIGN**

#### ***OVERVIEW***

The partition coefficient (*P*) is defined as the ratio of the equilibrium concentrations (*C<sub>i</sub>*) of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of *n*-octanol and water,

$$P_{ow} = \frac{C_{n-octanol}}{C_{water}}$$

*P*, therefore, is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (log *P*).

The partition coefficient of a substance between water and a lipophilic solvent (*n*-octanol) is one model variable that may be used to describe the transfer of a substance from the aquatic environment into an organism and the potential bioaccumulation of the substance. Studies show a highly significant relationship between the partition coefficient of different substances in the system water/*n*-octanol and their bioaccumulation in fish.

The following detail is provided as a guideline for the conduct of the study. Good scientific judgment will be used in conducting the study. The Study Director will document all changes in or revisions of this approved protocol. The actual procedure will be summarized in the final report.

#### ***SHAKE FLASK METHOD***

To determine a partition coefficient, equilibrium between all interacting components of the system must be achieved, and the concentrations of the substances dissolved in the two phases must be determined. This is accomplished by the thorough mixing of the two phases followed by their separation in order to determine the equilibrium concentration for the substance being examined.



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The Nernst partition law applies only at constant temperature, pressure, and pH for dilute solutions. It strictly applies to a pure substance dispersed between two pure solvents. If several different solutes occur in one or both phases at the same time, this may affect the results. Dissociation or association of the dissolved molecules results in deviations from the Nernst partition law. Such deviations are indicated by the fact that the partition coefficient becomes dependent upon the concentration of the solution. Because of the multiple equilibria involved, this test method should not be applied to ionizable compounds without corrections being made (The use of buffer solutions in place of water should be considered for such compounds).

The sensitivity of the method is determined by the sensitivity of the analytical procedure. This should be sufficient to permit the assessment of values of  $P_{ow}$  up to  $10^5$  when the concentration of the solute in either phase is not more than 0.01 mol/L. The substance being tested must not be water insoluble (mass concentration  $p > 10^{-6}$  g/L).

***CHEMICALS AND REAGENTS***

- Water, ASTM Type I, Double Distilled, RICCA Chemical or equivalent
- *n*-Octanol, HPLC grade, Sigma-Aldrich or equivalent

***EQUIPMENT***

- Balance
- Centrifuge, refrigerated
- pH Meter
- Temperature Monitoring by Ricerca Environmental Monitoring System (REMS)
- Standard laboratory glassware
- An Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES)

***PRESATURATION OF THE SOLVENTS***

The phases of the solvent system will be mutually saturated by shaking at the temperature of the experiment ( $25 \pm 1^\circ\text{C}$ ). Shake two large stock bottles of *n*-octanol and water for 24 hours on a mechanical shaker. Then let the solvents stand long enough to allow the phases to separate and to achieve a saturation state.

***PRELIMINARY TEST***

A preliminary test to estimate the partition coefficient and to develop an analytical method will be conducted. The volume ratio and quantities of substance to be used in the final test will be determined based on the preliminary test.

- The phosphorus content in the water layer determined using an ICP-AES
- The amount of the phosphorus in the octanol layer will be calculated by subtracting the amount of the phosphorus in the water layer from the total quantity of the phosphorus introduced. As the phosphorus content of K32 is 12.4% (determined by ICP in Ricerca Study No. 035469), the amount of total phosphorus introduced can be calculated.
- The preliminary assessment of the partition coefficient



- The minimum quantity of the test substance required for the analytical procedure
- The limitation of a maximum concentration in either phase of 0.01 mol/L

#### ***PREPARATION OF THE STOCK SOLUTION IN N-OCTANOL***

For a material balance during the test, a stock solution is prepared by weighing and dissolving of the test substance, K32, in *n*-octanol saturated with water. The nominal concentration of this stock solution will be calculated before it is employed in the determination of the partition coefficient.

#### ***TEST CONDITIONS***

The test temperature should be kept constant at  $25 \pm 1$  °C.

#### ***PERFORMANCE OF THE DEFINITIVE TEST***

*Establishment of the partition equilibrium* – Duplicate test vessels containing the required, accurately measured amounts of the two solvents together with the necessary quantity of the stock solution will be prepared for the test conditions. The *n*-octanol and water parts will be measured by volume. The entire volume of the two-phase system should nearly fill the test vessel. This will help prevent loss of material due to volatilization. Three tests are carried out in duplicate. In the first, the calculated volume ratio is added; in the second, twice the volume of *n*-octanol saturated with water is added; and in the third, half the volume of *n*-octanol saturated with water is added.

The test vessels will either be placed in a suitable shaker or shaken by hand. A recommended method is to rotate the centrifuge tube quickly through 180° about its transverse axis so that any trapped air rises through the two phases. Experience has shown that 50 such rotations are usually sufficient for the establishment of the partition equilibrium. To be certain, 100 rotations in 5 minutes are recommended.

*Phase separation* – In order to separate the phases, centrifugation of the mixture will be carried out. This will be done in a temperature-controlled laboratory centrifuge, or, if a non-temperature-controlled centrifuge is used, the centrifuge tubes will be re-equilibrated at the test temperature for at least 1 hour before analysis.

#### ***ANALYSIS***

An aliquot of the aqueous phase will be taken from each tube for each test condition and analyzed for the phosphorus content by ICP-AES. The pH of the water used and the aqueous phase for each of the samples will be measured.

The aqueous phase should be sampled by the following procedure to minimize the risk of including traces of the *n*-octanol: a glass syringe with a removable needle should be used to sample the water phase. The syringe should initially be partially filled with air. Air should be gently expelled while inserting the needle through the *n*-octanol layer. An adequate volume of aqueous phase is withdrawn into the syringe. The syringe is quickly removed from the solution and the needle detached. The contents of the syringe may then be used as the aqueous sample.



#### ***QUANTITATION OF THE PHOSPHORUS CONTENT IN TEST SAMPLES***

Quantitation of the phosphorus content in the test samples will be conducted by ICP-AES. The phosphorus content in the water layer determined using an ICP-AES. The amount of the phosphorus in the octanol layer will be calculated by subtracting the amount of the phosphorus in the water layer from the total quantity of the phosphorus introduced. As the phosphorus content of K32 is 12.4% (determined by ICP in Ricerca Study No. 035469), the amount of total phosphorus introduced can be calculated. The octanol-water partition coefficient (P) will be calculated based on the ratio of phosphorus present in both phases.

#### ***METHOD(S) FOR CONTROL OF BIAS***

In order to assure the precision of the partition coefficient, duplicate determinations will be made under three different test conditions, whereby the quantity of the substance specified, as well as the ratio of the solvent volumes may be varied. The determined values of the partition coefficient expressed as their common logarithms should fall within a range of  $\pm 0.3$  log units.

In addition, calibration data will be evaluated for accuracy within the working range of the quantitative measurements.

#### ***QUANTITATION OF RESULTS***

The following data will be calculated and reported:

1. The measured phosphorus content in aqueous phases for each determination. (This means that a total of 6 concentrations will be reported.)
2. The pH of the water used and of the aqueous phase during the experiment for each determination.
3. The weight of the test substance, the volume of each phase employed in each test vessel, and the total calculated amounts of phosphorus present in each phase after equilibration.
4. The calculated values of the partition coefficient (P), the mean for each set of test conditions, and the mean for all determinations. If there is a suggestion of concentration dependency of the partition coefficient, this will be noted in the report.
5. The standard deviation of individual P values about their mean.
6. The mean P from all determinations will also be expressed as its logarithm (base 10). The values of the partition coefficient expressed as their common logarithms should fall within a range of  $\pm 0.3$  log units.

#### ***PROPOSED STATISTICAL METHOD(S)***

Appropriate statistical methods for the analysis and evaluation of the experimental data will be used at the discretion of the Study Director. Common statistical methods used to evaluate the precision and accuracy of the measurements may include (as appropriate): average, mean, coefficient of variation, standard deviation, relative standard deviation, and confidence interval. Outlier measurements can be evaluated per standard statistical procedures such as the "t test" or "Q test."

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To improve data presentation and interpretation, and facilitate report preparation, the Study Director may apply computer programs for spreadsheets (e.g., Excel), graphics presentations (e.g., PowerPoint), and general standard statistics software.

## RECORDS TO BE MAINTAINED

Analysts shall document all experimentation such that an experienced scientist can reconstruct the work. Documentation shall include sample identifications, weighings, dilutions, calculations, etc. Additional documentation shall include instrumentation and equipment utilized during the study, as well as documentation of prepared reagents and solutions.

All study data shall be reviewed or verified and maintained in folders in the study activity file. Other comments, descriptions, calculations, correspondence, etc., shall be placed in the study activity file.

Upon conclusion of the study, copies of representative raw data (as appropriate), shall be submitted to the Sponsor. An accurate study file, including original raw data, shall be submitted to the Ricerca Biosciences, LLC Archives, 7528 Auburn Road, Concord, Ohio.

## REPORT

A final report will be prepared at the conclusion of the study. The report shall include, but not necessarily be limited to, the following:

- Name and address of the facility performing the study and the dates on which the study was initiated and was completed, terminated, or discontinued
- Reference(s) to, and/or a detailed description of, all methods used
- Representative data generated while conducting the study, and representative transformations, calculations or operations performed on the data
- Identification of the test, control, and/or reference substances used in the study
- All deviations and changes from the protocol
- A description of all circumstances that may have affected the quality or integrity of the data
- Name and signature of the Study Director, the names of other scientists or professionals, and the names of supervisory personnel involved in the study
- Statistical methods employed for analyzing the data. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis
- Locations where raw data and the final report are to be stored
- The signed and dated statement by the Ricerca Quality Assurance Unit specifying the dates of study inspections and dates the findings were reported to the Study Director and Management, when applicable

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- The signed and dated statement by the Study Director describing compliance with the Good Laboratory Practice Standards as specified in 40 CFR 160.

#### **AMENDMENTS AND DEVIATIONS TO THE PROTOCOL**

All agreed upon amendments will be expressed in writing, signed and dated by the Study Director. Protocol amendments will also be signed by the Sponsor, or the Sponsor may provide their approval by means of a Sponsor Approval Form. Copies of the signed amendments and any Sponsor Approval Forms will be returned to the Study Director and appended to the protocol.

Deviations from the protocol, if any, will be initiated by the Study Director, documented in the study file, and listed in the final report.



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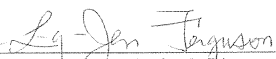
Protocol/K32 *n*-Octanol/Water Partition Coefficient  
Document Number: 035238-0


## PROTOCOL ACCEPTANCE


**Study Title:** Determination of *n*-Octanol/Water Partition Coefficient of K32


**Document Number:** 035238-0

**Testing Facility:** Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord, OH 44077

  
\_\_\_\_\_  
Ling-Jen Ferguson, Ph.D., Study Director  
Ricerca Biosciences, LLC

  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Robert McClanahan, Ph.D., Management  
Ricerca Biosciences, LLC

  
\_\_\_\_\_  
Date

***SPONSOR:*** KOCH AGRONOMIC SERVICES, LLC

***SPONSOR APPROVAL DATE:*** FEBRUARY 01, 2017

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## **PROTOCOL AMENDMENT ONE**

### **Study Title:**

Determination of *n*-Octanol/Water Partition Coefficient of K32

**Ricerca Document Number:** 035238-0-1

### **Data Requirements:**

US EPA OPPTS Test Guidelines 830.7550 Partition Coefficient (n-octanol/water),  
Shake Flask Method (August 1996)

**Testing Facility:**  
AgChem Product Development  
Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord OH 44077

**Study Sponsor:**  
Koch Agronomic Services, LLC  
2883 Miller Road  
Decatur GA 30035



*Changes to the original text are noted in bold font.*

## ORIGINAL PROTOCOL SECTION

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### **PRELIMINARY TEST**

A preliminary test to estimate the partition coefficient and to develop an analytical method will be conducted. The volume ratio and quantities of substance to be used in the final test will be determined based on the preliminary test.

- The phosphorus content in the water layer determined using an ICP-AES
- The amount of the phosphorus in the octanol layer will be calculated by subtracting the amount of the phosphorus in the water layer from the total quantity of the phosphorus introduced. As the phosphorus content of K32 is 12.4% (determined by ICP in Ricerca Study No. 035469), the amount of total phosphorus introduced can be calculated.
- The preliminary assessment of the partition coefficient
- The minimum quantity of the test substance required for the analytical procedure
- The limitation of a maximum concentration in either phase of 0.01 mol/L

## CHANGE TO

Pages 7

### **PRELIMINARY TEST**

A preliminary test to estimate the partition coefficient and to develop an analytical method will be conducted. The volume ratio and quantities of substance to be used in the final test will be determined based on the preliminary test.

- The phosphorus content in the water layer determined using an ICP-AES
- **The phosphorus content in the octanol layer determined using an ICP-AES**
- The preliminary assessment of the partition coefficient
- The minimum quantity of the test substance required for the analytical procedure
- The limitation of a maximum concentration in either phase of 0.01 mol/L



## ORIGINAL PROTOCOL SECTION

Pages 8

### *ANALYSIS*

An aliquot of the aqueous phase will be taken from each tube for each test condition and analyzed for the phosphorus content by ICP-AES. The pH of the water used and the aqueous phase for each of the samples will be measured.

The aqueous phase should be sampled by the following procedure to minimize the risk of including traces of the *n*-octanol: a glass syringe with a removable needle should be used to sample the water phase. The syringe should initially be partially filled with air. Air should be gently expelled while inserting the needle through the *n*-octanol layer. An adequate volume of aqueous phase is withdrawn into the syringe. The syringe is quickly removed from the solution and the needle detached. The contents of the syringe may then be used as the aqueous sample.

## CHANGE TO

Pages 8

### *ANALYSIS*

**An aliquot of each of the two phases will be taken from each tube for each test condition and analyzed by for the phosphorus content by ICP-AES.** The pH of the water used and the aqueous phase for each of the samples will be measured.

The aqueous phase should be sampled by the following procedure to minimize the risk of including traces of the *n*-octanol: a glass syringe with a removable needle should be used to sample the water phase. The syringe should initially be partially filled with air. Air should be gently expelled while inserting the needle through the *n*-octanol layer. An adequate volume of aqueous phase is withdrawn into the syringe. The syringe is quickly removed from the solution and the needle detached. The contents of the syringe may then be used as the aqueous sample.



## ORIGINAL PROTOCOL SECTION

Pages 9

### *QUANTITATION OF THE PHOSPHORUS CONTENT IN TEST SAMPLES*

Quantitation of the phosphorus content in the test samples will be conducted by ICP-AES. The phosphorus content in the water layer determined using an ICP-AES. The amount of the phosphorus in the octanol layer will be calculated by subtracting the amount of the phosphorus in the water layer from the total quantity of the phosphorus introduced. As the phosphorus content of K32 is 12.4% (determined by ICP in Ricerca Study No. 035469), the amount of total phosphorus introduced can be calculated. The octanol-water partition coefficient (P) will be calculated based on the ratio of phosphorus present in both phases.

## CHANGE TO

Pages 9

### *QUANTITATION OF THE PHOSPHORUS CONTENT IN TEST SAMPLES*

Quantitation of the phosphorus content in the test samples will be conducted by ICP-AES. The phosphorus content in the water layer determined using an ICP-AES. **The phosphorus content in the octanol layer determined using an ICP-AES.** The octanol-water partition coefficient (P) will be calculated based on the ratio of phosphorus present in both phases.

## ORIGINAL PROTOCOL SECTION

Pages 9

### *QUANTITATION OF RESULTS*

The following data will be calculated and reported:

1. The measured phosphorus content in aqueous phases for each determination. (This means that a total of 6 concentrations will be reported.)
2. The pH of the water used and of the aqueous phase during the experiment for each determination.
3. The weight of the test substance, the volume of each phase employed in each test vessel, and the total calculated amounts of phosphorus present in each phase after equilibration.
4. The calculated values of the partition coefficient (P), the mean for each set of test conditions, and the mean for all determinations. If there is a suggestion of concentration dependency of the partition coefficient, this will be noted in the report.
5. The standard deviation of individual P values about their mean.



6. The mean P from all determinations will also be expressed as its logarithm (base 10). The values of the partition coefficient expressed as their common logarithms should fall within a range of  $\pm 0.3$  log units.

## CHANGE TO

Pages 9

### *QUANTITATION OF RESULTS*

The following data will be calculated and reported:

1. **The measured phosphorus content in both phases for each determination. (This means that a total of 12 concentrations will be reported.)**
2. The pH of the water used and of the aqueous phase during the experiment for each determination.
3. The weight of the test substance, the volume of each phase employed in each test vessel, and the total calculated amounts of phosphorus present in each phase after equilibration.
4. The calculated values of the partition coefficient (P), the mean for each set of test conditions, and the mean for all determinations. If there is a suggestion of concentration dependency of the partition coefficient, this will be noted in the report.
5. The standard deviation of individual P values about their mean.
6. The mean P from all determinations will also be expressed as its logarithm (base 10). The values of the partition coefficient expressed as their common logarithms should fall within a range of  $\pm 0.3$  log units.

## REASON FOR CHANGE

The octanol phase of the test samples will be analyzed by ICP-AES for the phosphorus content.

## EFFECTIVE DATE

Date of Study Director's signature on the amendment.

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Protocol Amendment One/K32 *n*-Octanol/Water Partition Coefficient  
Document Number: 035238-0-1

## PROTOCOL AMENDMENT ONE ACCEPTANCE


**Study Title:** Determination of *n*-Octanol/Water Partition Coefficient of K32

**Document Number:** 035238-0-1

**Testing Facility:** Ricerca Biosciences, LLC  
7528 Auburn Road  
Concord, OH 44077

  
\_\_\_\_\_  
Ling-Jen Ferguson, Ph.D., Study Director  
Ricerca Biosciences, LLC

Mar. 13, 2017  
Date

  
\_\_\_\_\_  
Robert McClanahan, Ph.D., Management  
Ricerca Biosciences, LLC

13 March 2017  
Date

***SPONSOR:*** KOCH AGRONOMIC SERVICES, LLC

***SPONSOR APPROVAL DATE:*** MARCH 10, 2017

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## **APPENDIX B: K32 CERTIFICATE OF ANALYSIS**





## CERTIFICATE OF ANALYSIS

(Page 1 of 2)

**K32**

Batch/Lot No: **55700-30-13**

Ricerca Sample Code: **CS 22050**

Test	Test Method	Test Result
Active Ingredient Content and Identification	HPLC-UV TAN and LC-MS	See Table Below
Phosphorus Content	ICP	12.4%
Identification and Relative Quantification	<sup>31</sup> P-NMR	See Table Below
N-(n-butyl) thiophosphoric triamide (NBPT) Content	HPLC	20.04%
Conditions:	Refrigerate for prolonged storage (2-8 °C); may be handled at room temperature	
Study Initiation Date:	November 1, 2016	
Study Completion Date:	November 30, 2016	

### Active ingredient content by identification of peak using LC-UV TAN and LC-MS

Component	Observed Retention Time (minutes)	Observed %Area	Expected Molecular Mass	Observed molecular mass
1	6.58	0.2	478 and 406 [M+Na] <sup>+</sup>	406.1 [M+Na] <sup>+</sup>
2	6.98	1.9	334 [M+Na] <sup>+</sup>	334.1 [M+Na] <sup>+</sup>
3	7.48	4.2	262 [M+Na] <sup>+</sup>	262.1 [M+Na] <sup>+</sup>
3b	7.76	0.4		334.1 [M+Na] <sup>+</sup>
4	10.83	0.5	406 [M+Na] <sup>+</sup>	406.1 [M+Na] <sup>+</sup>
5	11.06	3.1	334 [M+Na] <sup>+</sup>	334.1 [M+Na] <sup>+</sup>
6	11.48	17.5	262 [M+Na] <sup>+</sup>	262.1 [M+Na] <sup>+</sup>
7	12.16	30.2	190 [M+Na] <sup>+</sup>	168.1 [M+H] <sup>+</sup>
8	12.62	0.2	406 [M+Na] <sup>+</sup>	406.1 [M+Na] <sup>+</sup>
9	12.89	0.4	334 [M+Na] <sup>+</sup>	334.1 [M+Na] <sup>+</sup>
10	13.55	0.1	252 [M+Na] <sup>+</sup>	274.1 [M+Na] <sup>+</sup>
11	17.4 to 30.0	41.1	Not Defined, mixture	Not Defined, mixture

### Identification and Relative Quantification using <sup>31</sup>P-NMR

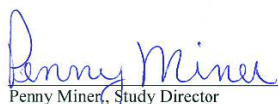
3.53 wt% K32	Chemical shifts (ppm)	NMR integral	Concentration (mM/g)*	Relative Ratio (to NBPT)
K32-a	[-0.22 - -0.61]	0.12	0.01	0.01
K32-b	[53.37 - 52.91]	0.33	0.03	0.02
K32-c	[55.73 - 55.43]	0.11	0.01	0.01
K32-d	[58.15 - 57.85]	0.15	0.01	0.01
NBPT	[59.80 - 59.49]	14.42	1.10	1.00
K32-e	[60.07 - 59.80]	7.83	0.60	0.54
K32-f	[60.31 - 60.07]	9.55	0.73	0.66
K32-g	[61.65 - 60.54]	5.48	0.42	0.38
L32-h	[64.97 - 63.38]	8.97	0.69	0.62
Total	[0.00 - 70.00]	46.96	3.60	

\*mMol of each compound in per gram of K32



## CERTIFICATE OF ANALYSIS

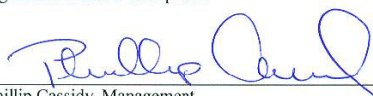
(Page 2 of 2)



Penny Miner, Study Director  
AgChem Product Development



Date



Phillip Cassidy, Management  
AgChem Product Development



Date

1. The objective of this study was to determine the active ingredient content, phosphorus content and mass spectra of K32, to be used as a test, reference or control substance in a study.
2. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b).
3. No deviations occurred from GLP regulations, the protocol, and relevant SOPs.
4. Data for this Certificate of Analysis is archived at the address below under Project Number 035469.
5. Only descriptive statistics were used.